

Statistically Analysing the Relationship between the Phosphate Levels and Nitrate Levels of Ponds and their Visible Biodiversity

Introduction and Background

The Environmental Protection Agency Report 'Water Quality in Ireland 2019-2024' reported that 48% of Irish surface waters were of unsatisfactory ecological status. The report highlighted excess nutrients in our surface waters as a major cause for ecologically damaged surface waters. Nationally, 32% of enclosed water bodies surveyed were of unsatisfactory ecological condition, and the same percentage had high phosphate concentrations.

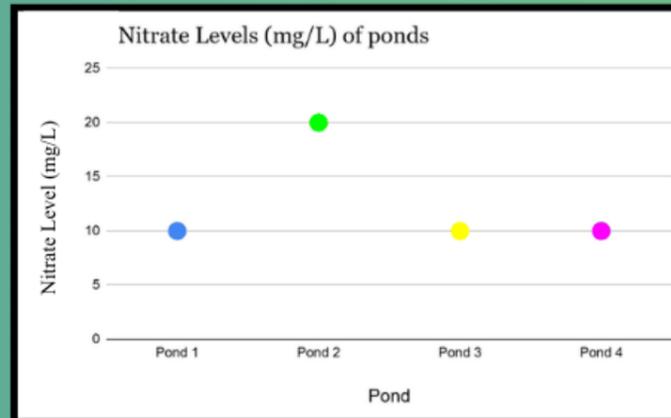
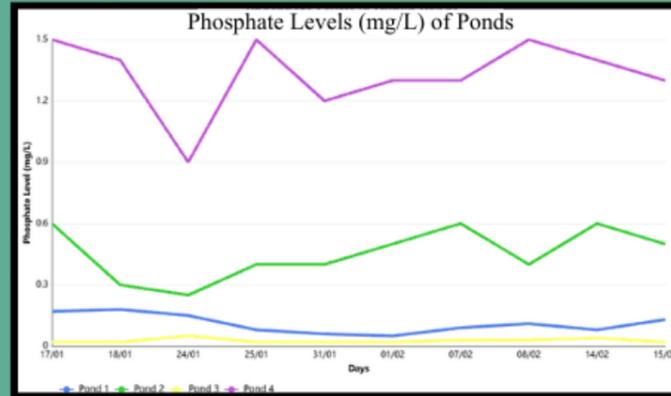
I decided to undertake this project after seeing this report published on RTE in December 2025. The report states that in order to resolve this issue, 'improved data sharing and integration between implementing bodies' could be utilised to raise awareness. Small ponds provide vital habitats for many critical species. According to the National Biodiversity Data Centre, ponds have demonstrated to host more biodiversity than rivers and lakes, yet over 50% of Ireland's wetlands and ponds have been lost over the last 100 years.

In surface waters, average phosphate concentration is 0.035 mg/L and average nitrate concentration is 10mg/L.

Sources: <https://www.epa.ie/publications/monitoring--assessment/freshwater--marine/EPA-Water-Quality-in-Ireland-Report.pdf>
<https://biodiversityireland.ie/>
<https://www.antaisce.org/Handlers/Download.ashx?IDMF=c98bbfaf-9ecb-4e32-b69f-6681f8b0e2f2>



Figure 1



The Control Variables

For this project, temperature was a control variable. I did the experiment at the same time each morning in order to try and keep the temperature as similar as possible. Time was also a control in this experiment- the data was collected between 9am and 10am each day.

Temperature °C	17/01	18/01	24/01	25/01	31/01	01/02	07/02	08/02	14/02	15/02
Pond 1	6	7	7	7	7	6	7	6	5	8
Pond 2	6	7	7	7	7	6	7	6	5	7
Pond 3	6	7	7	6	7	6	7	6	5	7
Pond 4	7	7	7	7	7	6	7	6	5	7

Objectives

- To determine if the phosphate levels in the ponds differ from each other
- To determine if the nitrate levels in the ponds differ from each other
- To determine if a relationship exists between the phosphate and nitrate levels of the ponds and the ponds' visible biodiversity
- To share the data I collected with public bodies to improve pond health and biodiversity

Limitations

- Error- For example, data collection error or data interpretation error.
- Bias- while every effort has been made to avoid bias, this project is limited to bias in data sampling, data analysis.
- Sample- a relationship determined based on these four ponds is limited to this sample and cannot be accurately inferred to apply to any/all ponds.

Method

This experiment was carried out 10 times in order to ensure accurate results for phosphate and nitrate levels in the pond. The four ponds were visited at a similar time each morning. Using a small jar I collected approximately 75ml of pond water. Each day I did the experiment I took a sample from a different place in the pond, because I was considering biodiversity from all around the pond. Using a plant identification app on my phone, I identified and recorded all plants around the pond. Any animal species I saw throughout the ten visits to the ponds were also identified and recorded. Temperature was recorded at each pond, which was always 6 or 7 degrees Celcius.

In order to measure phosphate levels, I used a freshwater phosphate test kit to carry out an acid phosphate gradient test. I added 5ml of sample pond water to a test tube. At a 45 degree angle five drops of PO4-1 reagent were added to the test tube, followed by nine drops of PO4-2 reagent. This created a colour which ranged from olive yellow to dark blue green (see figures 2,3). After 15 minutes the colour was observed and compared to a colour gradient chart and recorded. I repeated this process for all four sample waters from the ponds.

Using a nitrate test strip the nitrate levels of my samples were measured. This method first used a reducing agent (such as zinc) to reduce the nitrate to nitrite. The agent 'donates electrons', which chemically reduces the nitrate ions to nitrite ions. A Griess reaction then took place on the strip in order to produce a visible dye. In this process, nitrite reacted with sulfanilic acid on the strip, forming a diazonium salt. This salt immediately joined with another chemical, NED, which resulted in the formation of a pink to red azo dye. The amount of coloured dye produced is directly proportional to the nitrate concentration of the sample. The test strip was left for 30 seconds and then the colour was compared to a colour gradient chart. I recorded all my values in a Google Spread Sheet.

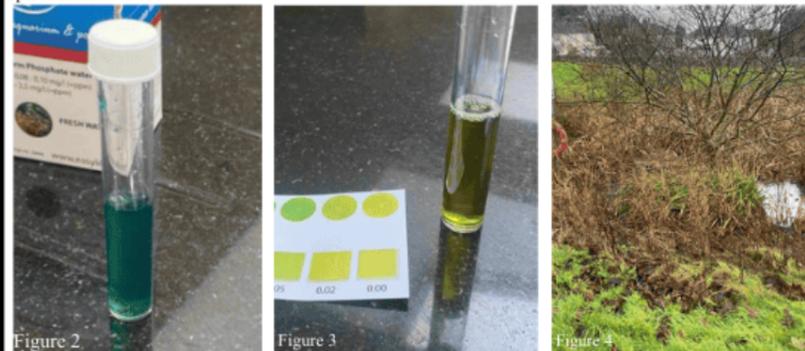


Figure 2

Figure 3

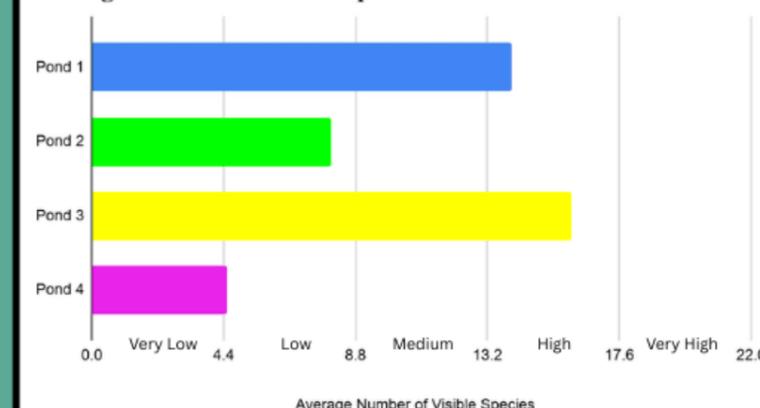
Figure 4

Visible Biodiversity

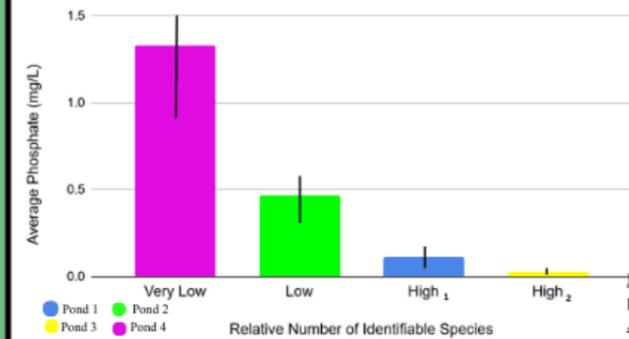
Visible biodiversity is the variety of life that is easily observable to the naked eye within a specific ecosystem. During my investigations, I counted all species I could identify every time I took a sample from a pond. I used a plant identifier app, PictureThis, to help with this process. Ponds are highly biodiverse ecosystems, with estimated between 50-300 species per pond, depending on how healthy they are. However, only approx. 5-20% of pond biodiversity is visible. For an average pond, (i.e one with 175 species) using this data I estimated that approximately 12.5% of these species would be visible, so approximately 22 species. However, since I didn't have the same resources when identifying new plants, I took this average as my relative 'very high' value.



Average Number of Visible Species



Average Phosphate (mg/L) vs. Relative Number of Identifiable Species



Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	10.6148275	3	3.538275833	301.008885	0	2.866265557
Within Groups	0.42317	36	0.01175472222			

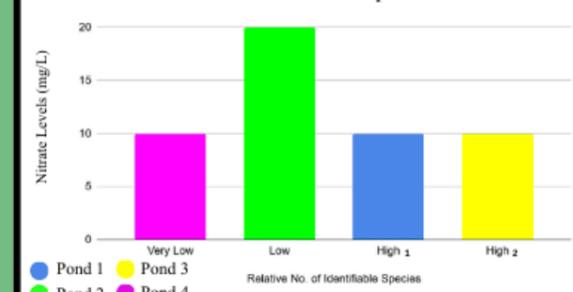
The Statistical Significance of Phosphate levels between Ponds

After observing a difference in the number of visible species, I carried out an ANOVA (analysis of variance) test to determine if there was statistically significant difference between the means of the phosphate level data collected on each pond. I accept the alternative hypothesis. For subsequent post-hoc testing, individual t tests were carried out on each group. Using a Bonferroni Correction, I identified a new p-value of 0.008. All tests hold as having a statistically significant difference between groups. This significant difference and the difference between the visible biodiversity indicates an inverse relationship between these variables.

Null Hypothesis: there is no statistically significant difference between the phosphate levels of the each pond
 Alternative Hypothesis: there is a statistically significant difference between the phosphate levels of each pond

T Tests between Phosphate Levels of Ponds	P-value
Pond 1 vs Pond 2	0.00000035256
Pond 1 vs Pond 3	0.00026029621
Pond 1 vs Pond 4	0.00000000134
Pond 2 vs Pond 3	0.00000029639
Pond 2 vs Pond 4	0.00000000265
Pond 3 vs Pond 4	0.00000000295

Nitrate Levels (mg/L) vs Relative Number of Identifiable species



Nitrate Levels Statistical Observations

*Nitrate levels didn't vary between measurements
 From the graph opposite and the table below it is evident there is little variation between the groups. Since they all had the same nitrate levels, except for an outlier, pond 2, this variable does not follow the relationship observed with the phosphate levels and visible species data.

Groups	Count	Sum	Average	Variance
Column 1	10	100	10	0
Column 2	10	200	20	0
Column 3	10	100	10	0
Column 4	10	100	10	0

Conclusions

Objective 1: To determine if phosphate levels in the ponds differ from each other
 Following my data collection and data analysis I determined there was a significant statistical difference between the phosphate levels in all the ponds, (p=0), as concluded from the Analysis of Variance Test (single factor ANOVA) and subsequent t tests.

Objective 2: To determine if nitrate levels in the ponds differ from each other
 Following my data collection and subsequent data analysis I could not determine a significant statistical difference between the nitrate levels of my ponds. With the exception of one outlier, (Pond 2, 20mg/L), all ponds appeared to have equal nitrate levels. Through further investigation I identified a possible cause for this, a factory spill that occurred near the pond not long ago. However, given the equipment I had available it was difficult to determine exact nitrate levels. If I was to do this project again I would use more accurate equipment.

Objective 3: To investigate if a relationship exists between phosphate and nitrate levels of the ponds and the ponds' visible biodiversity.
 From my data analysis and interpretation, a relationship was determined. It was determined that in the ponds with very low or low visible biodiversity, the highest phosphate levels occurred consistently. In the ponds with a high visible biodiversity, the lowest phosphate levels occurred consistently. This was expected, as excess phosphate can be harmful to ponds. No relationship between nitrate levels and visible biodiversity could be concluded.

Excess levels of phosphate in water bodies can cause harmful processes such as eutrophication. This occurs when these nutrients are present in excess quantities, making them food for algae. Algae grow and reproduce quickly, forming an algal bloom. This bloom absorbs sunlight, meaning that light can't reach plants at the bottom of the pond causing them to die. As algae uses up all the nutrients for food, it will also die. Bacteria starts to break down dead plants and algae and as it does so it releases more nutrients back into the water. Bacteria consume oxygen as they grow and respire, causing more plants and other organisms to die.

Objective 4: to share the data I collected with public bodies to improve pond health and biodiversity.

Pond 4 had very high phosphate levels and low levels of biodiversity. In appearance it was very overgrown, and some algae was visible on the surface. I got in contact with the Local Authority Water Programme following my project to investigate what I could do in order to improve the health of this pond.